

Anopheles (Cellia) dirus, a New Species
of the *Leucosphyrus* Group from Thailand
(Diptera: Culicidae)¹

E. L. Peyton² and Bruce A. Harrison³

ABSTRACT. *Anopheles (Cellia) dirus*, a new species of the *leucosphyrus* group, and a probable major vector of malaria in Thailand, is described and illustrated in the adult, pupal and larval stages. Comparisons are made between this species and *An. (Cel.) balabacensis* Baisas, the name, which until now, has been commonly applied to *dirus*.

INTRODUCTION

With the resurgence of malaria in many parts of the Oriental region, there is renewed interest in basic studies on the vector species of *Anopheles* and in particular, the *leucosphyrus* group. This is especially true in Thailand, where results of several significant studies have been published and others are in progress. Some of the more significant Thailand reports dealing with the species of the *leucosphyrus* group, heretofore treated as *balabacensis* *balabacensis* Baisas, are: Scanlon and Sandhinand (1965); Gould, Scanlon and Ward (1966); Scanlon, Peyton and Gould (1967); Rutledge, Gould and Tantichareon (1969); Chin (1969, 1970); Ismail, Notananda and Schepens (1974, 1975) and Wilkinson et al. (1978). In addition, there are several unpublished reports of research conducted since 1964 by personnel of the U. S. Army Medical Component, Southeast Asia Treaty Organization (SEATO), presently Armed Forces Research Institute of Medical Sciences (AFRIMS), Bangkok. These studies have demonstrated that the species treated as *balabacensis* (= *dirus*) in Thailand is a probable major vector of malaria wherever it occurs and in some areas it may be the only vector (Scanlon and Sandhinand 1965:68; Chin 1970:14).

The *leucosphyrus* group is currently being revised by the senior author and a more comprehensive treatment of *dirus* and the other members of the group will appear later. However, in view of the significance of the Thailand species as a suspected vector of malaria and of the studies that are now being conducted in Thailand, we feel that a preliminary description of this species is now required.

¹This work was supported by Research Contract No. DAMD-17-74-C-4086 from the U. S. Army Medical Research and Development Command, Office of the Surgeon General, Washington, D. C. 20314.

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Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE 1979		2. REPORT TYPE		3. DATES COVERED 00-00-1979 to 00-00-1979	
4. TITLE AND SUBTITLE Anopheles (Celia) dirus, a New Species of the Leucosphyrus Group from Thailand (Diptera: Culicidae)				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Armed Forces Research Institute of Medical Sciences,U. S. Army Medical Component,APO,AP,96346				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution unlimited					
13. SUPPLEMENTARY NOTES					
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15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Same as Report (SAR)	18. NUMBER OF PAGES 13	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

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FEMALE. Essentially as described for *balabacensis*, differing primarily in the wing, and possessing the usual group characteristics of, legs with numerous pale spots, a long white band at apex of hindtibia and a slightly shorter band on base of hindtarsomere 1 producing a very conspicuous continuous white band over tibiotarsal joint; wing vein Cu_1 with a distinct bend on basal 0.5 and vein M bending in opposite direction at same point. Diagnostic features as follows: *Head.* Proboscis 1.5-2.3 mm., ratio to forefemur 1.03-1.08; palpus 1.4-2.3 mm., ratio to proboscis 0.93-1.00, ratios of length of segments: 3/5, 2.2-2.7, 3/4, 1.3-1.7, segments 2-4 with narrow apical pale bands, 5 with a broader apical pale band varying from 1.00-2.60 the length of the preapical dark band. *Thorax.* Propleural setae 1:1-3:3 usually (64/100) 2:2. *Wing* (Fig. 1, with 5 examples of variation on costa, subcosta and radius). Dark and light spots on most veins except costa (C), subcosta (Sc) and radius (R) highly variable in number, length and placement; costa without basal pale (BP) spot, rarely without prehumeral pale (PHP) or humeral pale (HP) spots, sector pale (SP) occasionally reduced to very small spot, but usually rather prominent, presector dark (PSD) spot on R with 1-4 pale interruptions, usually 2, the spot almost always extending basally beyond corresponding dark spot on C on one or both wings (94/100), usually reaching HD or beyond on one or both wings (83/100), and occasionally reaching to middle of HD or beyond on one or both wings (21/100), extension sometimes represented by a small dark spot of 2-4 scales at HD, C without accessory sector pale (ASP) spot (100/100), Sc without ASP, rarely with 1-4 pale scales on one or both wings (8/100), ASP always present on R, usually small, occasionally reduced to 3-5 pale scales only, dark spot on R basal to ASP 0.25-3.00 length of ASP, usually 1.0 or more, occasionally with a small pale interruption on one or both wings (31/100); apex of wing pale from apical dark (AD) to apex of vein R_2 followed by a short dark spot between R_2 and R_3 and a longer pale spot extending to a little past R_{4+5} . *Legs.* Apical pale band of hindtibia without dark scales extending into basal portion; hindtarsomere 4 with a distinct basal pale band or patch, band usually complete but frequently reduced or incomplete on ventral side, always prominent on dorsal side at least on one leg, 5 sometimes pale at base. *Abdomen.* Tergum VI usually with a very few narrow light brown scales on apical margin, VII always with a few light or dark brown scales on apical margin, VIII profusely covered with golden scales and setae on apical 0.5; cercus with similar scales and setae dorsally and laterally; sternum VI occasionally with a very few light brown scales on apical margin, VII with a dense patch of erect brownish black scales

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on apical margin, VIII with lateral patches of sparse creamy golden scales, occasionally with a few brown scales intermingled.

MALE. Essentially as in female but wing generally paler and scaling reduced. *Head.* Proboscis longer than female about 2.4 mm., ratio to forefemur 1.38-1.50. *Thorax.* Propleural setae 1:1-2:2 usually 2:2. *Wing.* PSD of R almost always with basal extension on at least one wing, usually on both (63/66), usually reaching to HD or beyond on at least one wing (57/66), with 1-3 pale interruptions, usually 2, C without ASP, Sc usually without ASP, rarely (17/66) with 1-4 pale scales present and usually only on one wing, occasionally pale on ventral side, ASP always present on R and usually slightly longer than female, basal dark spot of $\frac{1}{2}$ SD 0.00-2.00 of ASP and less frequently with extra pale interruption. *Abdomen.* Resembles female, scales on terga VI, VII when present, usually pale, tergum VIII (which is usually ventral in mature specimens) with numerous brown scales on apical 0.5, sternum VIII (usually dorsal) profusely covered with creamy white scales, basimeres clothed with similar scales on sternal and lateral surfaces and a few dark brown scales basolaterally.

PUPA (Fig. 2). Chaetotaxy as figured. Diagnostic features as follows: *Abdomen.* Seta 1-III with 5-9 branches, 5-III with 5-11 branches; 1-IV with 4-8 branches, 5-IV with 5-8 branches, 9-IV of short type, length (40 specimens) 0.030-0.059 mm., ratios of length of seta 9, IV/III 1.50-3.19 (\bar{x} =2.14), IV/V 0.28-0.53 (\bar{x} =0.41); 1-V with 2,3 branches, rarely (1/40) single, 5-V with 4-7 branches; 1-VI usually double (1,3), 5-VI with 4-8 branches; 1-VII single to triple, 5-VII with 4-6 branches.

LARVA (Fig. 3). Chaetotaxy as figured. Diagnostic features as follows: *Head.* Seta 2-C single, simple or with 2-4 fine lateral barbs, 4-C posterolaterad of 2-C, single or bifid, usually projecting forward to near or slightly beyond the bases of 2-C, 13-C with 4-8 branches. *Thorax.* Seta 1-P with 11-18 branches, stem strong but usually not noticeably flattened and expanded basally, inserted on a large sclerotized basal tubercle which is joined with an equally developed basal tubercle of 2-P, each with a strong apical tooth or spine projecting forward over the bases of each seta, teeth varied in shape but usually slightly longer and pointed on base of 1-P, 14-P with 4-7 branches, very rarely with 8 branches on one side; 14-M with 6-8 branches; 3-T with 4-6 weak branches, rarely with weakly developed leaflets. *Abdomen.* Seta 1-I small, with strong base and 4-9 rigid branches, 3-I usually bifid or trifid (1-3), 9-I usually with 3,4 (3-5) branches; 1-II weakly developed, with 7-18 branches, without differentiated lanceolate leaflets and except for slightly longer and greater number of branches, not significantly different from 1-I; 2-IV,V usually with 4-6 branches, rarely with 3 on one or both segments, 13-IV usually with 3 branches (2-3) greater than 0.5 length of 10-IV; 1-VII smaller than 1-VI with 10-16 moderately broad lanceolate leaflets, rarely 1-3 leaflets may exhibit weak apicolateral serrations; pecten teeth usually with 4,5 long teeth and 7-11 noticeably shorter teeth of varied lengths.

TYPE-DATA. Holotype female with slide of pupal and larval skins, with the following collection data: THAILAND, *Prachin Buri*, Ban Bu Phram, 21 July 1971, Kol Mongkolpanya and team, collectors, collection number 06042-1, SEAMP accession number 327, collected as a larva from an elephant footprint in

secondary scrub-forest at an elevation of 80 m. Allotype male with slide of pupal and larval skins, same data as holotype, number 06042-2. Paratypes: 5 females, 2 males (6 with pupal and/or larval skins on slides), same data as holotype, collection numbers 06042-5, -6, -7, -100, -101, -102, and 1 fourth stage larva on slide; other paratypes with same data as holotype except as follows: 1 male (1p slide), collection number 06022-11; 2 females, 28 July 1971, collection number 06047, collected from small ground pool at 100 m; 1 male, 28 July 1971, collection number P12, collected from a small ground pool at 150 m; 1 male (p slide), Ban Tablan, 20 July 1971, collection number 06033-100, collected from trunk of large (fallen?) tree at 80 m; 1 female (1p slide), Ban Tablan, 27 July 1971, collection number 06037-1, collected from small ground pool at 80 m; 3 females, 1 male (1 lp, 2 p slides), Ban Tablan, 29 July 1971, collection numbers 06053-1, -100, -101; 4 females (1 lp, 1 p slides), Ban Tablan, 17 August 1971, collection numbers 06064, -2, -100; 1 female (1p slide), Ban Tablan, 7 September 1971, collection number 06088-1; 2 females (2 lp slides), Ban Tablan, 7 September 1971, collection numbers 06089-9, -10; 2 females, 2 males (1p slide), Ban Tablan, 20 July 1971, collection numbers P3 and P3-102.

The holotype, allotype and paratypes are deposited in the National Museum Natural History. Paratypes of 2 females and 2 males with associated slides of immature stages will be deposited in the British Museum (Natural History).

DISTRIBUTION. Probably represents most records of "*balabacensis*" on mainland Southeast Asia, north of 8° latitude but restricted here to confirmed Thailand records pending further study of material from other Southeast Asian countries.

A total of 238 ♀, 167 ♂, 272 p, 142 l, and 198 L from 202 separate collections were examined from the following provinces in Thailand: Chanthaburi, Chiang Mai, Chon Buri, Chumphon, Kanchanaburi, Krabi, Lampang, Mae Hong Son, Nakhon Nayok, Nakhon Ratchasima, Nakhon Sawan, Nakhon Si Thammarat, Nan, Phangnga, Prachin Buri, Ranong, Songkhla, Tak and Trat.

The above merely reflects the provinces in which studies have been concentrated and from which specimens were preserved and deposited in a major museum or the Medical Entomology Project collection. It may be safely assumed that this species will occur in most provinces where stands of forest remain, except possibly a few of the drier plains areas of the central and northeastern parts of the country and the extreme southern provinces adjoining Malaysia.

DISCUSSION. The identity of the nominal subspecies *balabacensis balabacensis* presents a very complex and confusing systematic problem. According to the previously accepted concept, it was the most widely distributed member of the *leucosphyrus* group occurring throughout most of the Oriental region including, Philippines (Palawan-Balabac), northeastern Borneo (Sabah), Java, Peninsular Malaysia (northwestern corner, state of Perlis), Thailand, Cambodia, Laos, Vietnam, Taiwan, Burma, Bangladesh, India (Assam and Karwar), People's Republic of China (Hainan and Yunnan) and Sri Lanka; but there are some obvious peculiarities in this distribution. Colless (1956) found that *balabacensis*

probably did not occur in western Borneo south of Miri, and was apparently replaced by pure populations of *leucosphyrus* Doenitz west of this line. The nominal subspecies *balabacensis* does not occur in central and southern Peninsular Malaysia where it seems to be replaced by a common form which Colless (1957) named, *balabacensis introlatus*. It occurs only in the extreme northwestern state of Perlis which extends considerably north of the eastern Thailand-Malaysian border. Everything north of this line is supposedly *balabacensis*. *Anopheles balabacensis* extends west into Burma, Assam and Bangladesh; there is a great gap across central India to the southwestern coastal state of Mysore where it is again reported. The form occurring in Taiwan was described as *takasagoensis* by Morishita (1946) but was later synonymized with *balabacensis* by Colless (1956). In the Philippines it occurs on the southwestern islands of Balabac (type-locality) and Palawan only. In Luzon it is replaced by *balabacensis baysasi* Colless 1957. Colless (1956-1957) recognized the Fraser's Hill form from Pahang, Peninsular Malaysia and a Negros form from the island of Negros, Philippines, plus a number of other minor varieties of local populations cited in the introduction of this paper.

It is not our purpose to now attempt to address all of these problems, but each will be investigated more thoroughly as our studies progress.

Although the adults of *dirus* and *balabacensis balabacensis* are very similar in appearance and the wing characters are highly variable, the 2 are easily recognized except in an extremely small percentage (~5%) of specimens. Similar variation is often seen in the wing of several other currently recognized species or subspecies. Figure 1 shows 2 examples of *balabacensis* and 5 of *dirus* of variation on costa, subcosta and radius of wing.

The presector dark spot on wing vein R is variable both in the number of pale interruptions and in the basal extension of the spot beyond the corresponding dark spot on the costa in most recognized forms of *balabacensis*. However, except in *balabacensis sensu stricto* and *dirus*, the frequency of a basal extension is extremely low as in the case of Taiwan specimens where a basal extension was seen in only one of 37 specimens. A basal extension of this spot is characteristic in *dirus* where it extends anywhere from slightly beyond the corresponding spot on the costa on one wing only to as far as the base of the humeral dark spot of costa on both wings. The common condition in *balabacensis* is no extension or only extended to the level of the presector pale spot. Colless (1956) found about 65% of specimens from Borneo without any extension but 4/5 Philippine specimens with a slight extension. In 36♀ and 13♂ from Balabac and Palawan we observed the following: no extension 20/49 (41%); slight extension, one or both wings but only into presector pale spot level 19/49 (39%); to level of humeral dark spot or beyond on one or both wings 10/49 (20%). In 44♀ and 23♂ from Sabah, Borneo the following was observed: no extension 40/67 (60%), slight extension but only into presector pale spot level 20/67 (30%), to level of humeral dark spot or beyond 7/67 (10%). While this character alone will not separate the 2 species it does show a higher degree of development in *dirus* and this in combination with the development of the accessory sector pale spot on costa and subcosta should separate at least 95% or better of specimens.

The most significant difference in the adult stage of the 2 species is in the development of the accessory sector pale (ASP) spot. In specimens of *dirus* there is never an ASP on the costa. In *balabacensis*, Colless (1956) found an ASP present on the costa on one or both wings in 67/112 (60%) females examined from Borneo, Balabac and Java, but in a local Labuan Island population he found it present on one or both wings in 88% of 50 specimens examined and on 20 specimens from mainland coastal Borneo and 30 from the interior he found it present 70% and 60% respectively. Our examination of 80♀ and 36♂ specimens, 49 from Balabac and Palawan and 67 from Sabah, Borneo, found an ASP on C present on one or both wings in 85/116 (73%). In *dirus* an ASP is also undeveloped on the subcosta, except for a few specimens with 1-4 pale scales in this position which are usually on one wing only and most commonly 1,2 scales 25/166 (15%). It is difficult to extrapolate this character from the Colless (1956) study of *balabacensis* since he combined it with the presence of ASP on R. However, it is well developed on our specimens from Balabac, Palawan and Borneo in the female and particularly prominent in the male. The actual proportion of specimens with an ASP on the subcosta of both wings was 113/116 (97%) with only one of the remaining three specimens resembling the condition found in *dirus*. The ASP on R is also usually longer in *balabacensis*, with the dark spot on R basal to ASP 0.00-1.00 length of ASP and without an extra pale interruption. Therefore, a well developed ASP on the subcosta of both wings or an ASP on the costa of one or both wings should separate 95 percent or better of specimens and this character with others noted should assure separation of adults with a considerable degree of confidence.

The most striking difference in the 2 species occurs in the pupal stage, which does not often provide good characters for separation of species within the group. It has been known for some time that the group could be partially divided by the length of seta 9-III-V which was usually expressed as "long or short" type for 9-IV. Colless (1956-1957) presented these in actual measurements and ratios of length of IV/III and IV/V. Only *balabacensis balabacensis* and *balabacensis introlatus* had a long seta 9-IV with *balabacensis* showing a ratio for IV/V of 0.67-0.87 with an average of 0.78. Our examination of 40 specimens of *dirus* and 28 specimens of *balabacensis* from Balabac, Palawan and Borneo of seta 9-IV shows the following: for *dirus* length of 0.030-0.059 mm. (0.043 mm.), ratios of length IV/III 1.50-3.19 (2.14), IV/V 0.28-0.53 (0.41), for *balabacensis* length 0.056-0.089 mm. (0.074 mm.), ratios of length IV/III 3.05-5.54 (4.11), IV/V 0.65-1.05 (0.81).

There are a few fairly consistent larval differences in the 2 species. Seta 1-II in *balabacensis* is weakly palmate, usually with 9-18 distinct narrow lanceolate leaflets, a rather expanded basal stem, light pigmentation and noticeably better developed than 1-I. Seta 2-IV,V is 3,4 branched in 26 specimens from Balabac, Palawan and Borneo. Only 4 specimens had 4 branches on one side only and never on both segments of the same specimen. It appears that these characters will separate approximately 80% of the larvae of *dirus* and *balabacensis*.

BIONOMICS. *Anopheles dirus* is typically an inhabitant of foothills, forests or forest fringes, but will also invade orchards and plantation environments. It is most abundant during the rainy season when the immature stages can be found in a great variety of small, shallow, temporary, water-holding depressions in the soil, provided it receives sufficient shade. The pools may occasionally have considerable organic matter and/or may be quite turbid, but if the water is not freshened frequently by rain the species will not be encountered. In Thailand the favored habitat appears to be elephant footprints, especially in forest with heavy logging operations. The species does not occur in grassy pools or the larger semipermanent forest pools. The immatures have been collected from the following habitats: elephant footprints, pools in dry stream beds, pools at edge of streams and springs, rock pools, ground pools, seepages, wheel ruts, pits dug for mining gems, bamboo stumps, human and domestic animal footprints, bases of large fallen tree trunks, and longitudinal depressions in hollow logs. The adults will feed readily on man but usually feed late at night, with peak activity often occurring between midnight and 0300 h (Scanlon and Sandhinand 1965). In some areas biting activity begins as early as 1900 h, with an increase around 2100 h and lasting through 0300 h for outdoor collections, and between 2200-0400 h for indoor collections (Wilkinson et al. 1978). Both of the above papers should be read for a more complete review of the biology and the role of *An. dirus* in the transmission of malaria.

ACKNOWLEDGMENTS

We wish to thank Ronald A. Ward, Michael E. Faran and Douglas J. Gould for critically reviewing the manuscript. We are particularly indebted to Rampa Rattanarithikul and Kol Mongkolpanya of the Entomology Department, AFRIMS, Bangkok, for their considerable technical assistance in collecting, preparing and examining material from Thailand. Special appreciation is expressed to Ann L. Hoskins Dery and Vichai Malikul for preparation of the drawings and to Lotte B. Schiff and Sharon Harrison for the typing of the manuscript.

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balabacensis

Fig.1

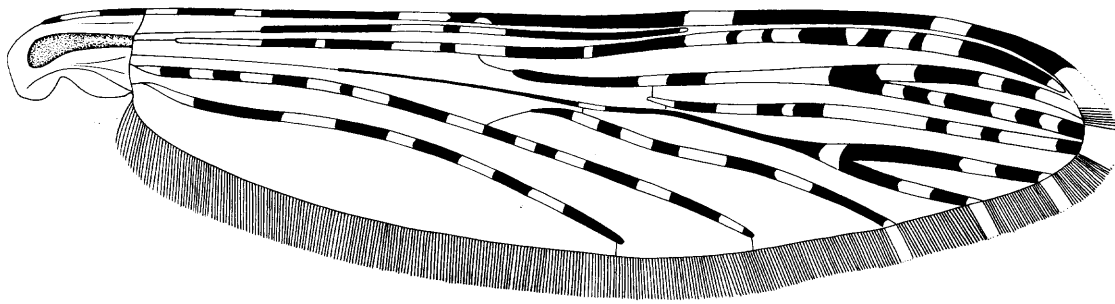
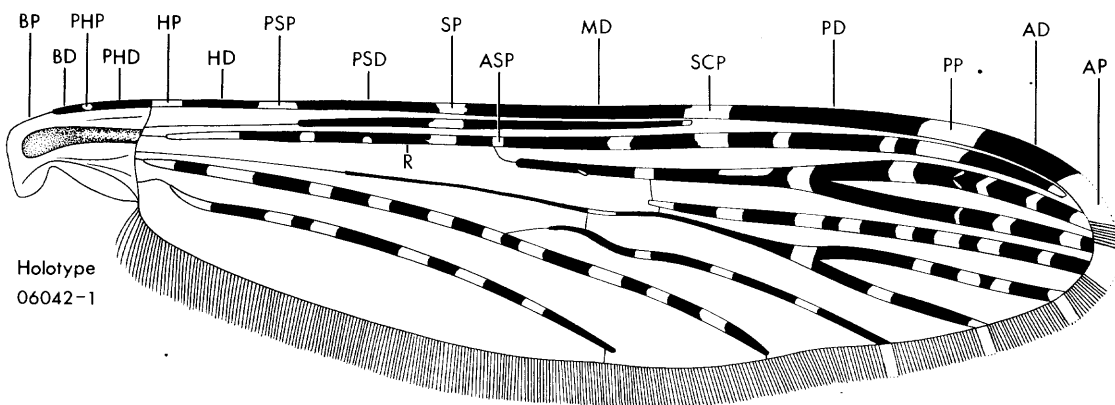
*dirus**Ichai Mahikul*

Fig. 2

CT

1.0

sp

dirus (Seta 9-II-VII)

III II

IV

V

VI

VII

0.1

balabacensis (Seta 9-II-VII)

III II

IV

V

VI

VII

IX

P

♀

dirus

A. H. Dery

dirus

Fig.3

